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Neutrophil–lymphocyte ratio as an early predictor for patients with acute paraquat poisoning
A retrospective analysis

Zong Xun Cao, MD, Ya Qi Song, MBBS, Wen Jing Bai, MBBS, Wen Jie Wang, MD, Yong Zhao, MBBS, Su Li Zhang, MBBS, Shun Yi Feng, MD*

Abstract

This retrospective study aimed to investigate whether the neutrophil–lymphocyte ratio (NLR) can be used as an early predictor of 90-day survival in patients with acute paraquat (PQ) poisoning.

This study enrolled 105 patients with acute PQ poisoning admitted from May 2012 to May 2018. Kaplan–Meier curve, receiver operating characteristic curve, and Cox proportional hazards regression analyses were used to investigate the predictive value of NLR for 90–day survival of patients with acute PQ poisoning.

The 90-day survival rate was 40.95% (43/105). Survivors had lower NLR ($P<.001$), which was an independent predictor of 90-day survival according to the Cox proportional hazard regression analyses. The area under the NLR curve was 0.842 (95% CI: 0.767–0.917, $P<.001$) in predicting 90-day survival.

Our findings showed that low NLR was a valuable early predictor of 90-day survival in patients with acute PQ poisoning.

Abbreviations: AUC = area under the curve, NLR = neutrophil–lymphocyte ratio, PQ = paraquat, ROC = receiver operating characteristic.

Keywords: neutrophil–lymphocyte ratio, paraquat, prognosis

1. Introduction

Paraquat (N, N′-dimethyl-4,4′-bipyridinium dichloride; PQ) is a rapid-acting, nonselective herbicide that has been used for weed control in underdeveloped agricultural countries. PQ kills plants rapidly by deactivating the photosynthetic mechanism. It also has a considerable toxicity toward humans and has become the leading cause of death by pesticide poisoning.[1] PQ also has considerable toxicity toward animals and humans and has widely been utilized for suicide. In humans, an oral dose of >30 mg/kg damages the lungs and kidneys, thereby leading to subsequent death.[2] Although various treatment modalities for acute PQ poisoning exist, the fatality rate remains high, with affected

individuals presenting a mortality rate of 50% to 90%.[3–7] Identifying the factors associated with early mortality may provide primary clinical information for correct evaluations and treatment decisions. For example, early prediction of inevitable death is important to terminate inappropriate treatments in terminal acute PQ poisoning patients. In addition, effective prediction methods can help clinicians to determine the conditions and severity of PQ poisoning to patients and their family. Therefore, confirming PQ-poisoning diagnosis and risk assessment in a timely manner is particularly important.

Many studies reported to date indicated that PQ concentrations, especially plasma PQ concentrations, are highly associated with the prognosis of PQ poisoning; the measurement of plasma and urine PQ concentrations has been confirmed as the most useful approach in PQ poisoning test. High-performance liquid chromatography can accurately determine PQ concentrations but requires extremely expensive, technical, and accurate equipment that are unavailable in most hospitals. This situation raises the need to develop a valuable predictor for prognosis to guide future therapeutic intervention.

One of the simplest and most readily available tests in the clinic is the complete blood cell count, which reports the absolute neutrophil and lymphocyte counts. The serum neutrophil-to-lymphocyte ratio (NLR) is an economical and convenient indicator of systemic inflammation. NLR is a useful prognostic indicator in various diseases, including community-acquired pneumonia,[8] ischemic heart disease,[9] ulcerative colitis,[10] appendicitis,[11] and cancer.[12] However, limited data[13] suggested that NLR can be a predictor for prognosis in patient with PQ poisoning. Thus, this retrospective clinical study investigated the early predictive value of NLR in patients with acute PQ poisoning.

Table 1

General characteristics upon arrival between survival and mortality groups.

	Mortality group (*n*=62)	Survival group (*n*=43)	*P*
Age, yr	42.00 (32.00)	33.00 (21.00)	.093
Gender, male/female	26/36	19/24	.819
Time from ingestion to gastric lavage, h	1.00 (1.00)	1.00 (1.50)	.410
Time from ingestion to blood sampling, h	1.00 (1.00)	1.00 (1.50)	0.424
Alanine aminotransferase, ALT, U/L	32.65 (16.78)	27.00 (9.60)	.015
Creatinine, μmol/L	104.50 (68.50)	62.00 (22.00)	<.001
Alveolar oxygen partial pressure (PaO$_2$, mmHg)	89.97 ± 10.62	93.37 ± 9.27	.093
Plasma paraquat concentration, ng/mL	3.65 (9.00)	0.30 (0.90)	<.001
NLR	12.47 (9.92)	5.41 (4.65)	<.001

NLR = neutrophil–lymphocyte ratio.

2. Methods

2.1. Ethics and consent

This retrospective clinical study was approved by the Ethics Review Committee of the Cangzhou Central Hospital, China (No. 2017-090-01). The medical records of all PQ poisoning patients hospitalized from May 2012 to May 2018 in the emergency department were reviewed, and all data collected were anonymized and standardized. Considering that this study involved a retrospective review of existing data, specific written informed consent was obtained from patients. However, informed consent regarding the treatment risk following acute PQ poisoning was obtained from all patients upon their initial admission.

2.2. Patients

Patients were diagnosed with acute PQ poisoning by checking their plasma PQ concentrations. The inclusion criteria included:

1. patients aged >14,
2. patients with PQ poisoning through oral intake,
3. hospital admission within 12 hours of poisoning, and
4. no history of serious chronic disease.

Exclusion criteria included:

1. dermal or intravascular exposure,
2. patients with other pesticide poisoning,
3. pregnant patients,
4. cases with infection,
5. cases with immunosuppressive therapy, or
6. cases with blood systemic diseases.

2.3. Data collection

All data, which included:

(1) demographic parameters, such as age and gender;
(2) time interval from PQ ingestion to gastric lavage in the emergency department;
(3) plasma PQ level;
(4) time from ingestion to blood sampling, and
(5) clinical laboratory parameters including NLR, alveolar oxygen partial pressure (PaO$_2$), creatinine, alanine aminotransferase (ALT), and plasma PQ concentration, were collected by experienced physicians by using a standard collection form in a Microsoft Excel spreadsheet.

The primary endpoint of the study was 90-day survival, and survival time was identified from medical records or telephone follow-up.

2.4. Sample size

To test the hypothesis that NLR has 80% sensibility and 80% specificity, with alpha error of 0.05, power of the study of 80%, and allowable error of 12.5%, we should have 99 patients as the sample size.[14] However, we decided to enroll 105 patients to allow for probable dropouts.

2.5. Statistical analysis

All statistical analyses were performed with Statistical Product and Service Solutions version 13.0 software (SPSS, Chicago, IL). The results were presented as the means±standard deviations and assessed using the 2-independent sample t-test or 1-way ANOVA when the data fitted a normal distribution. Otherwise, the results were presented as medians and interquartile ranges, which were

Table 2

General characteristics upon arrival stratified according to NLR level.

|	NLR <10 (*n*=53)	NLR 10–20 (*n*=40)	NLR >20 (*n*=12)	*P*
Gender, male/female	21/32	17/23	7/5	.496
Time from ingestion to gastric lavage, h	1.00 (1.25)	1.00 (1.50)	1.00 (0.50)	.610
Time from ingestion to blood sampling, h	1.00 (0.88)	1.00 (1.50)	1.00 (0.50)	.526
Alanine aminotransferase, ALT, U/L	28.60 (12.85)	28.20 (13.93)	32.85 (12.33)	.423
Creatinine, μmol/L	69.00 (49.00)	81.00 (37.00)	134.00 (86.00)	.009
Alveolar oxygen partial pressure (PaO$_2$, mmHg)	92.10 (12.25)	89.60 (16.98)	85.75 (7.18)	.217
Plasma paraquat (PQ) concentration, ng/mL	0.90 (2.60)	2.95 (5.55)	1.95 (6.25)	<.001

NLR = neutrophil–lymphocyte ratio.
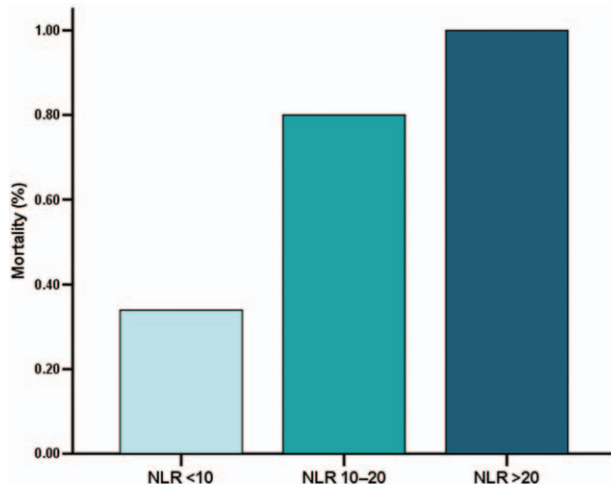
Figure 1. Mortality of the groups according to the NLR level. NLR = neutrophil–lymphocyte ratio.
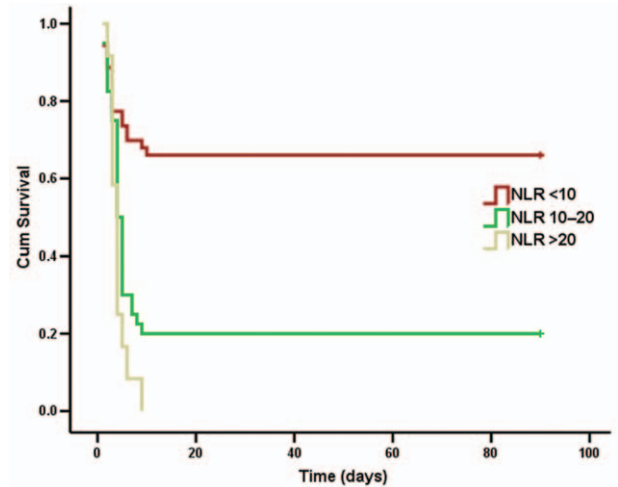

Figure 2. Kaplan–Meier analysis of survival curves for the groups according to the NLR level. NLR = neutrophil–lymphocyte ratio.

assessed using the 2- or multiple independent sample and nonparametric tests, respectively. Categorical variables were presented as absolute frequency and percentage and compared using Pearson's chi-squared test. Kaplan–Meier method was used to establish survival curves, and the survival differences were compared using log-rank test. Cox proportional hazard regression models were applied to establish univariate and multivariate survival analyses in evaluating the independent predictive factors. $P < .05$ in the univariate analysis was required for a variable to enter the multivariate model. The analyses of the receiver operating characteristic (ROC) curves and the area under the ROC curves (AUC) were performed to evaluate how well the NLR functioned as a predictive value for the mortality in patients with PQ poisoning. The P values were 2-tailed, with $P < .05$ considered as statistically significant.

3. Results

3.1. Patient characteristics

Overall, 105 acute PQ poisoning patients were identified from May 2012 to May 2018, with more women (60, 57.1%) than men (45, 42.9%). The 90-day survival rate was 40.95% (43/105), and the medial survival time of dead cases was 4.03 (1.00–10.00) days. At the baseline, non-survivors had high NLR, ALT, creatinine, and plasma PQ concentrations (Table 1). When stratified according to NLR (<10, 10–20, and >20), the creatinine and plasma PQ concentrations upon arrival significantly differed among groups (Table 2), and the case-fatality rates were 34% in NLR <10, 80% in NLR 10 to 20, and 100% in NLR >20 (Fig. 1).

Table 3

Cox regression model.

|	Univariate COX model HR (95% CI)	P	Multivariate COX model HR (95% CI)	P
Age, yr	1.013 (0.998–1.027)	.084	N/A	
Gender, male/female	0.916 (0.553–1.518)	.735	N/A	
Time from ingestion to gastric lavage	1.010 (0.849–1.202)	.910	N/A	
Plasma paraquat concentration	1.068 (1.049–1.088)	<.001	1.051 (1.023–1.080)	<.001
Alanine aminotransferase (ALT)	1.020 (1.003–1.037)	.024	1.014 (0.995–1.034)	.146
Creatinine	1.019 (1.014–1.025)	<.001	1.013 (1.005–1.021)	.001
Alveolar oxygen partial pressure (PaO$_2$)	0.982 (0.957–1.008)	.176	N/A	
NLR	1.050 (1.030–1.070)	<.001	N/A	
<10	reference		reference	
10–20	3.197 (1.778–5.750)	<.001	3.529 (1.912–6.516)	<.001
>20	5.001 (2.356–10.616)	<.001	3.208 (1.248–8.246)	.016

N/A = not applicable.

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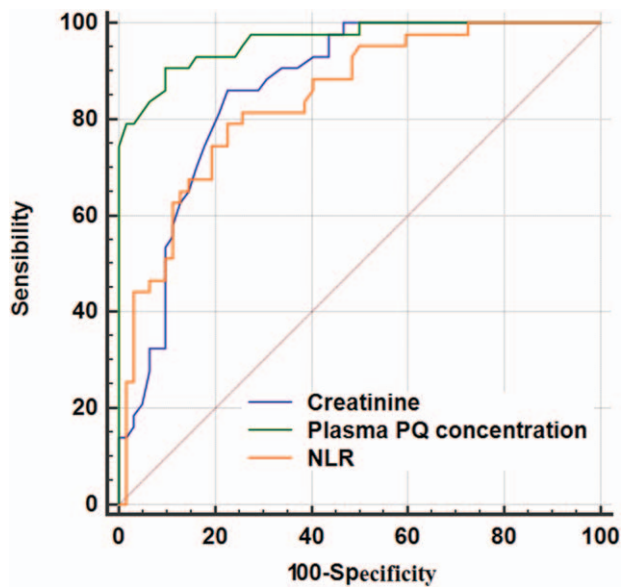
Figure 3. Area under the receiver operating characteristic curve analysis. NLR = neutrophil–lymphocyte ratio, PQ = paraquat.

3.2. Kaplan–Meier survival analysis and Cox proportional hazard regression analyses

The Kaplan–Meier survival curves (Fig. 2) showed that low NLR was associated with high 90-day survival (log-rank test; $P < .001$). Low NLR, ALT, creatinine, and plasma PQ concentrations were associated with 90-day survival in the univariate Cox proportional hazard regression analyses. Meanwhile, low NLR, creatinine, and plasma PQ concentrations were associated with a 90-day survival in the multivariate Cox proportional hazard regression analyses (Table 3).

3.3. Correlation analysis

Correlation analysis demonstrated that NLR was negatively correlated with survival time ($r = -0.409$; $P < .001$) and 90-day survival ($r = -0.410$; $P < .001$).

3.4. ROC curve analysis for 90-day mortality

The ROC curves of NLR, creatinine, and plasma PQ concentrations showed an AUC value of 0.842 (95% CI: 0.768–0.906, $P < .001$), 0.863 (95% CI: 0.783–0.923, $P < .001$), and 0.964 (95% CI: 0.908–0.990, $P < .001$), respectively (Fig. 3 and Table 4). The predictive values of plasma PQ concentration were significantly higher than those of creatinine ($Z = 2.720$, $P = .007$) and NLR ($Z = 3.229$, $P = .001$). By contrast, the

predictive values of NLR were similar to those of creatinine ($Z = 0.422$, $P = .673$).

4. Discussion

PQ poisoning can cause severe multiple organ failure involving the kidneys, liver, lungs, adrenal glands, and the central nervous system. The lethal toxicity of PQ has resulted in a high mortality rate in the range of 60% to 80%,[3,4,6,15] which has been attributed to PQ's inherent toxicity and the lack of any effective treatment to ameliorate the toxic effects of poisoning. The 90-day survival rate was 40.95% in our study, which was consistent with the results of previous reports.[3,4,6,15]

Neutrophil remarkably increased after PQ poisoning, whereas lymphocyte levels declined. The molecular mechanisms underlying PQ toxicities have been unidentified. However, several potential mechanisms may be summarized as follows. First, the activities of proinflammatory cytokines, such as interleukin-6, 8, and 17, remarkably enhanced within hours after PQ ingestion and rapidly promoted the extensive influx of neutrophils.[16–19] Second, PQ could accelerate the generation of reactive oxygen species and significantly reduce neutrophil apoptosis through nuclear factor-κB, p38 mitogen-activated kinase, and myeloid cell leukemia sequence 1.[20,21] Meanwhile, intracellular redox state imbalance caused lymphocyte apoptosis by activating apoptotic enzyme caspase-3.[22–25]

In the present study, multiple factor COX regression analysis revealed that initial plasma PQ, creatinine, and NLR concentrations were associated with the risk of mortality from PQ poisoning. A rise in creatinine is a good predictor because it is both an indicator of the extent of ongoing toxicity and of the ability to eliminate PQ.[26,27] However, creatinine cannot be used as a predictor for acute PQ poisoning in patients with chronic renal function impairment. The most effective way to assess the severity of acute PQ poisoning is by monitoring plasma PQ level. The plasma level peaks early, that is, at 1 hour after PQ ingestion, followed by a rapid decline with a steep gradient due to rapid distribution from circulation to other compartments.[28] The plasma PQ level obtained within h after PQ ingestion is a reliable predictor for prognosis. The role of prediction gradually diminished over time.[29]

Consistent with the results of a previous report,[13] NLR is a valuable early predictor of 90-day mortality in patients with acute PQ poisoning. However, the NLR had an area of 0.842 in our study, which was lower than the value (0.916) suggested by the previous report.[13] We speculated that the discrepancy is related to the time response. The estimated mean interval time from PQ ingestion to the blood test in our study is 4 hours, whereas 7 hours was used in a previous study. Neutrophil count was still declining, and that of lymphocyte was increasing; NLR did not peak within 4 hours.

Table 4

ROC curve analysis.

Variable	Area under ROC curve	95% CI	Cutoff	Sensitivity (%)	Specificity (%)	Youden index
Plasma paraquat concentration	0.964	0.932–0.996	1.55	90.3	0.907	0.81
Creatinine	0.866	0.794–0.932	74.5	77.4	86.0	0.634
NLR	0.842	0.767–0.917	8.73	77.4	79.1	0.565

N/A = not applicable.

This study has several limitations that should be acknowledged. First, retrospective studies that use administrative data may contain unintended bias. Retrospective study design does not allow comprehensive data collection that is directly related to the study question. Second, the relatively small sample size our study may have prevented the statistical detection of clinically significant differences and thus led to limited generalizability. Third, differential diagnosis of NLR in various diseases mainly relies on oral pesticide history and plasma PQ test.

In conclusion, our findings showed that low NLR was a valuable early predictor of 90-day survival in patients with acute PQ poisoning. For hospitals that lack facilities to measure plasma PQ concentration, NLR monitoring provides additional useful information in selecting the therapy and assessment of prognosis. In addition, NLR monitoring features the advantages of low cost, stability, rapid testing, and repeatability. However, sophisticated studies are needed to identify the NLR potential further.

Author contributions

Conceptualization: Zong Xun Cao, Shun Yi Feng.
Data curation: Zong Xun Cao, Ya Qi Song, Wen Jing Bai, Yong Zhao, Su Li Zhang.
Investigation: Ya Qi Song, Wen Jing Bai, Yong Zhao, Su Li Zhang.
Writing – original draft: Zong Xun Cao, Wen Jie Wang.
Writing – review & editing: Shun Yi Feng.

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